ON THE HÆMATOZOAN INFECTIONS OF BIRDS

BY

W. G. MacCALLUM, M. D., Johns Hopkins University

From
THE JOURNAL OF EXPERIMENTAL MEDICINE
Vol. III, No. 1, 1898

ON THE HÆMATOZOAN INFECTIONS OF BIRDS.

By W. G. MacCALLUM, M. D. (Johns Hopkins University).

Plate XII.

Our knowledge of the nature of these infections, sometimes loosely designated as avian malaria, is mainly due to the researches of Danilewsky, Laveran, Kruse, Sakharoff, Grassi and Feletti, Celli and Sanfelice, Pfeiffer, Di Mattei, and Labbé. The only work upon this subject so far done in America was reported in the papers on the etiology and pathology of hæmatozoan infections in American birds, read by Opie and myself before the Medical Society of the Johns Hopkins Hospital in November, 1896, and printed in brief in the *Bulletin of the Johns Hopkins Hospital* for March, 1897. These papers, which are published in full in the present number of this Journal (pp. 79 and 103), give the results of observations made at the suggestion of Dr. Thayer of the Johns Hopkins Hospital, in the summer of 1896, on the blood and organs of various birds.

During the past summer (1897) I have continued the work in Dunnville, Ontario, confining my attention mainly to crows, which seem to afford the most promising field for observation.

Many species of birds have been found to be infected with these organisms to a greater or less degree—in Europe sparrows, finches, starlings, blackbirds, crows, hawks, etc. In Ontario and Maryland, where we have procured our birds, we have found sparrows, blackbirds, owls and crows infected, and of these the crows were most frequently so, for although the number is too small to furnish any definite conclusions as to relative frequency of infection, some idea of this may be gained from the fact that of eighty sparrows the blood of five showed organisms, while of twenty-one crows sixteen were infected.

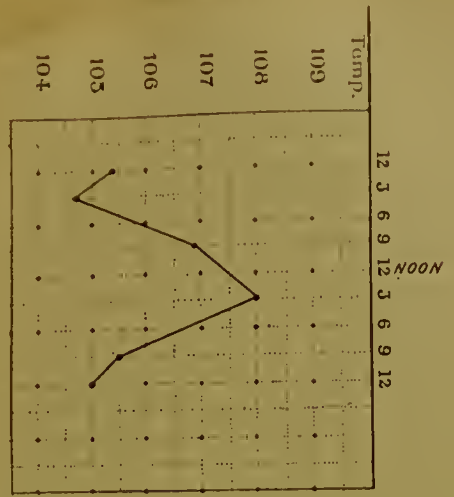
The organisms in these cases are very similar in many respects to those found in the blood of human beings suffering from malaria.

There are, however, certain well-marked differences which I shall briefly review before detailing the results of my work of the past summer. According to the observations of Labbé,* and those of Opie,† two types of organisms may be distinguished differing in form and life history as well as in the effects produced on the host. Of these the first, named by Labbé "Halteridium," begins its cycle of existence as a small oval or rounded hyaline body, situated laterally or at one end of the nucleus of the red corpuscle. As it increases in size it extends in a direction conditioned by the position of the nucleus, at the same time by its metabolic activity forming in itself granules of blackish-brown pigment. As it grows it extends more and more along the sides of the elliptical nucleus until finally, on reaching its adult stage, it is seen to coil about the nucleus, embracing it quite closely and expanding at each end into a wider portion into which the pigment, which increases with the age of the organism, is often gathered. Stained specimens show a tingeable cytoplasm in which the pigment is embedded and within which lies a clear non-stainable body with a central stained spot. This clear body is thought to be the nucleus with central nucleolus, and plays an important part in the process of segmentation as described by Labbé. He finds that the nucleus of the parasite divides into two segments, which separate and proceed to the ends of the organism, where they divide into multiples of two, thus forming two rosettes of segments lying at the ends of the red corpuscle. In the middle of the red corpuscles beside the nucleus there remains the pigment together with some debris of the organism. Opie has not been able to confirm this observation, but has seen the formation of single rosettes in the bone-marrow. I have also found forms which might be designated as pre-segmenting in the circulating blood. In these the organism had rounded itself off in one end of the corpuscle, the pigment being gathered into a mass in its centre, the margin showing a number of indentations corresponding with which there were the fine refractive dots thought to represent the nuclear flecks in each segment.

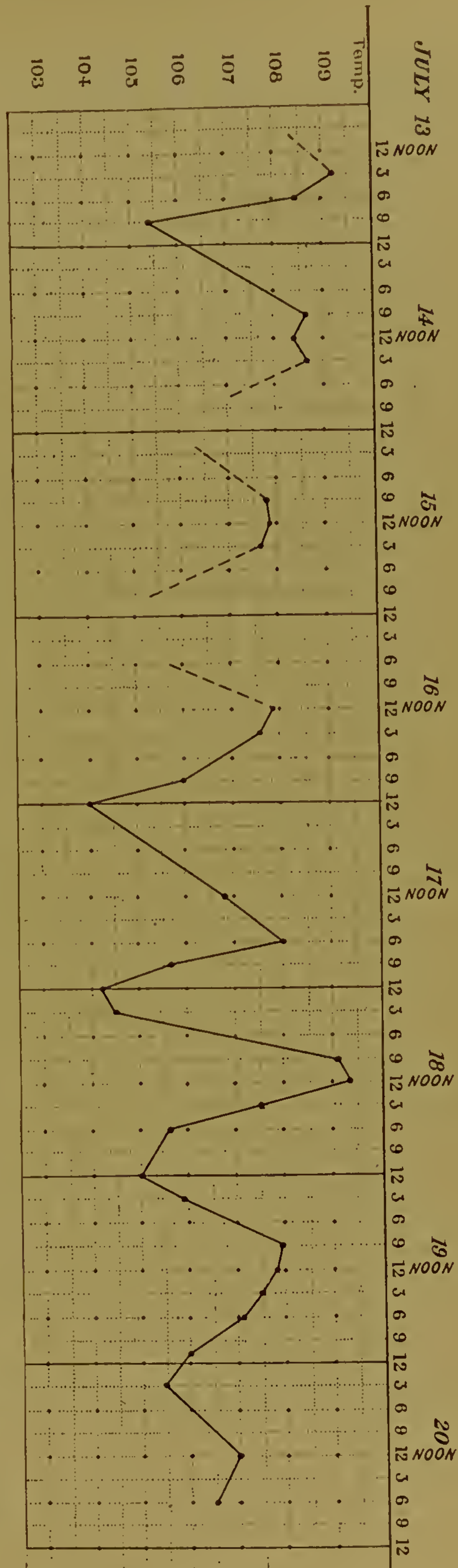
* *Arch. de zool. expér.*, série 2, 1894.

† The present number of this Journal, p. 79.

Chart I, showing Daily Temperature of a Normal Crow.



Temperature Chart II of Crow infected with Halteridium.*



Flagellation almost identical with that observed in the case of the human malarial organisms is very readily seen in these organisms. The exact length of the cycle is very difficult to determine, because all the organisms present in the blood of any bird do not segment at the same time, nor even, so far as can be determined, in large groups, but at any time almost all stages can be seen. Labbé places the cycle at seven to eight days. He, as well as Danilewsky,† states that very little can be observed clinically in infections with this form of organism, although in the case of the second form about to be described, there are febrile symptoms with a certain rise in temperature. I have attempted to construct the accompanying chart from a series of observations on the temperature of a crow infected with the Halteridium and, although it does not extend over any great length of time, it will serve to give some idea of the course of the temperature as compared with that of a crow which showed no infection. There is seen to be a daily rise of from 3 to 5

* Kindly charted for me by Miss Hutchinson of the Training School for Nurses of the Johns Hopkins Hospital.

† *Arch. f. Hyg.*, xxv (1895), 227.

degrees from the temperature recorded at midnight, and this, to a certain extent, is true of the normal crow, so that it can hardly be ascribed to the effect of the organisms, but we must perhaps rather consider the crow to be, to a certain extent, poikilothermic.

There is, however, a rather higher temperature recorded on July 13, 18, etc., suggesting that some group of considerable size might be segmented at intervals of five days. This, however, is not very constant and may have been accidental, although the blood on these days of high temperature seemed to show a larger percentage of hyaline forms.

A variety of this Halteridium is found in the crow, blackbird, etc., in which the organism is much more slender, and when full-grown finds room to lie in the corpuscle without touching the nucleus.

The second type of organism, named by Labbé "Proteosoma," also begins its cycle as a small, rounded or ovoid, hyaline body, generally situated near one end of the red corpuscle. As it grows, instead of accommodating itself to the position of the nucleus, it causes such a change in the stroma of the corpuscle that the nucleus no longer retains its central position, but is pushed to the opposite end of the corpuscle, where it comes to lie transversely. The pigment, which is on the whole finer and blacker than that of the preceding form, makes its appearance early and is distributed over the body of the parasite, which, in its adult stages, takes on a rounded or irregular polygonal form. Flagellation occurs precisely as in Halteridium. Segmentation takes place in the circulating blood and results in the formation of a simple rosette with a central mass of pigment. As stated above, this form of infection seems to be accompanied by more serious clinical phenomena than the other.

The Proteosoma I had no opportunity of observing during the past summer, and it is more especially of the Halteridium, which occurs so abundantly in crows, that I here wish to speak. The infections with the latter organism are generally relatively slight—one or two organisms being found in a slide of blood—and the birds may show no external evidence of being sick. There are other cases, however, where the bird can be recognized at a distance as being sick by its evident weakness, its uncertain flight and altered voice, as well as its draggled ap-

pearance and lustreless plumage. Such birds often show an enormous infection, at times more than a third of the corpuscles being invaded, with sometimes two or even three parasites in a single corpuscle. They may, however, recover to a certain extent in confinement when well fed, and the organisms may be seen to diminish in number most markedly.

On examining a slide of fresh blood where the organisms are numerous, examples of flagellating forms are observed with extreme readiness about two and a half minutes after the blood has been taken from the bird (Plate XII, Fig. 6). This process, accurately described by most authors on the subject, takes place as follows: The adult organism is seen to draw itself together into a sphere within the red corpuscle, and sometimes immediately, but more often after a short delay, it begins to be greatly agitated, the pigment dancing about and the surface of the sphere taking on an active undulating motion which lasts but a short time, for the organism suddenly bursts from the corpuscle, scattering the remains of the latter, and in its place beside the nucleus of the corpuscle, which now lies free in the plasma, it throws out four or more flagella, which thrash about wildly and sooner or later become detached and wriggle away. The sphere is much reduced in size by this throwing out of flagella and the pigment is concentrated, although occasionally a pigment grain is included in one of the flagella, which are not perfectly regular in form, but may branch or run into one another at the base. Their number is not constant and may change in the process of their formation. The remains of the sphere continue to be agitated and, after the loss of the flagella, its pigment sets up a most active dancing. Often it constricts itself into two or more parts, which may reunite, and sometimes a more than usually stout prolongation of protoplasm, which has failed to become a perfect flagellum, may adhere and beat about for a long time. Disintegration and death are the inevitable fate of these remains of the flagellate body, even if it escapes for any length of time one of the voracious leucocytes which wander about.

There is a certain variation in the relation of the act of throwing out flagella to the extrusion from the corpuscle. At times the organ-

ism can be seen to throw out flagella while still within the corpuscle, these beating the nucleus about within the enveloping membrane of the corpuscle, which is violently agitated and distorted like a loose bag containing the struggling organism. At last the membrane bursting liberates the fully developed flagellate form. In other cases the sphere slowly escapes through a rent in the capsule, and after a period of quiescence outside the corpuscle, begins to be agitated and finally to put forth its flagella.

Other organisms escaping quietly in this way through a rent in the capsule remain quiescent and never become flagellated (Plate XII, Fig. 5).

There is always in these processes the escape of the remaining hæmoglobin of the corpuscle into the plasma, with a corresponding blanching of the corpuscle and, although the thin capsule is left for a time, it soon becomes invisible.

Many theories as to the nature of these flagellated forms and the significance of the process have been put forward, none of which have met with general credence. Laveran thought the flagella were pre-existent in the body of the intracorpuscular parasite. He believed the flagellate forms of the human malarial organism to be the final and most perfect stage of the parasite, and that they developed within the round and crescentic bodies which he regarded as cysts. Dock considered that they represented a resting stage of the organism capable of existing independently, perhaps even of reproducing themselves, but also capable under favorable circumstances of reproducing a typical growth of the parasite. Mannaberg suggests that they are organs which permit the parasite to enter into a saprophytic existence. "I suspect," he says, "that the flagellate bodies enter upon the first stages of a cycle of existence outside of the human body, and that as a result of the unfit culture medium the death of the young spores occurs." The whole Italian school believes them to be due to degenerative changes. Manson, as is well known, has advanced the idea that the flagellate bodies represent the forms in which the parasite exists outside the human body, that the flagella penetrate from the stomach into the body of mosquitos which have sucked the blood of

infected human beings, and that, after a further unknown process of development, they come again, through the water in which the mosquitos deposit their eggs and die, into the human body. Sakharoff recognizes the flagellate bodies as dying forms; he finds the large red nucleus (with Romanowsky's stain) with a clustered chromatic filament. In the free forms this last breaks up into several filaments, which leave the parasite as flagella—a perversion of karyokinesis which is provoked by physico-chemical means. Zacharias claims to have produced flagella in epithelial cells by the action of phosphoric acid, and Labbé gives it as his opinion that the process of flagellation resembles a chemical one, that lack of oxygen hastens the process, as also does the sudden drop in temperature from 42 to 15 degrees C. Flagellate forms, he concludes, are abnormal agony products preceding degeneration, which do not exist in the living blood, but form outside the body under the diverse physico-chemical influences which modify the plasma and corpuscles obtained from the vessels.

It will be seen that none of these ideas, except that of Sakharoff, are based on any observations, but are for the most part pure hypotheses. That the flagellate forms are the agony products of the dying parasites seems to be the most generally accepted idea, and this, it is true, has the fact to support it that it occurs only under unfavorable circumstances, and is followed by the death of the organisms.

Other motile bodies have from time to time been described as occurring in the blood of birds. Danilewsky * describes elongated non-pigmented organisms found in fresh blood plasma. These are seen to swell and contract and frequently to advance in a curved or spiral line. He thinks they are related to the Drepanidia, but Labbé suggests with more probability that they are free flagella.

In addition to these Danilewsky also describes an organism resulting from the transformation of an intra-corpuscular pigmented form. This becomes extra-corpuscular and spherical and, after the lapse of ten to thirty minutes, a process appears at the periphery of the body which, increasing in size, gives rise to a curved elongated body which he calls a vermiculus. This body, which has acquired a nucleus,

* Danilewsky, Parasitologie comparée du sang. Kharkow, 1889.

moves about in a curved line in the plasma. Kruse * described a similar form in the blood of a crow, and Pfeiffer † in that of an owl. No further idea as to their nature was given.

It was noted by Opie (p. 92) that there were variations in the adult form of the Halteridium, which I have endeavored to depict in Plate XII, Figs. 1 and 2 and Figs. 3 and 4. This difference, which sharply distinguished two forms of the organism, consisted in a granular appearance in the one as compared with the clear hyaline protoplasm of the other forms. The protoplasm in the first form was not simply finely granular, but in a groundwork of finely granular protoplasm could be seen minute hyaline points. This difference was much more sharply brought out by staining with methylene blue when the granular form took on a deep stain, the fine granules especially appearing dark blue while the hyaline points remain wholly unstained. The hyaline form, on the other hand, took almost no stain, the slight tinge of blue being for the most part peripheral, sometimes with a blue fleck in the centre of the unstained portion. The pigment granules are, as a rule, finer in the granular forms than in the clear hyaline forms.

Opie (p. 96) put forward the suggestion that it might be these clear non-staining forms which become flagellated, the granular forms being the ones observed to degenerate without flagellation.

My observations have enabled me to confirm this distinction, and to demonstrate that it is true that the granular forms never become flagellated and that the clear forms alone do so. Indeed, it may be prophesied of any organism while yet intra-corpuscular that it will or will not become flagellated.

While watching one of these specimens which had stood for some time I was surprised to see an elongated organism moving slowly across the field followed by others moving in all directions. These were evidently modifications of the organism familiar in fresh specimens, and the question of greatest interest was to show their relation to the other forms. They corresponded very closely with the vermi-

* Kruse, Virchow's *Archiv*, cxx (1890), 541, and cxxi, 359.

† Pfeiffer, *Zeitschr. f. Hyg.*, viii (1890), 309.

culi of Danilewsky, and I have assured myself since that they are identical with them.

As the forms which flagellated were always observed to disintegrate or succumb to a leucocyte, the idea remained that the motile forms might arise from the forms described as being extruded from the corpuscle without flagellation. On carefully watching these for fifteen to twenty minutes it proved to be easy to see the change from the non-motile sphere into the motile elongated organism or vermiculus. In one or two instances, however, it was noted that such non-motile spheres apparently became flagellated without the extreme agitation usually seen in the process of flagellation, and without any diminution in their bulk, and later on gave rise to the motile forms. This seemed to contradict the idea that these arose exclusively from the non-flagellating forms, and apparently there remained the conclusion that flagellation was a process which proceeded more or less violently in different cases; that where the violence was great the store of energy of the organism was exhausted; where, however, it did not take place, or took place in such a modified and mild form that not much energy was expended, the organism was able to take on a motile form.

I decided, however, on the impulse of another idea, to observe carefully in the same field a granular form and a hyaline form from the time of extrusion from the corpuscle to the beginning of the motile stage, and, having found such a field, the following picture presented itself: The two forms lay at some distance from one another separated by the plasma and a few corpuscles. The granular form happened to escape from the corpuscle first and lay perfectly quiet beside the free nucleus and the shadow of the corpuscle. Soon the hyaline body, becoming greatly agitated, burst from the corpuscle and threw out active flagella, which beat about for a few moments and finally tore themselves loose. Then came the acme of the process. One of the four flagella passed out of the field, but the remaining three proceeded directly toward the granular form, lying quietly across the field, and surrounded it, wriggling about actively. *One of the flagella, concentrating its protoplasm at one end, dashed into the granular sphere,*

which seemed to put out a process to meet it, and buried its head, finally wriggling its whole body into the organism, which again became perfectly round. The remaining flagella, seeking to repeat this process, were evidently repulsed, and soon became inactive and degenerated. Immediately on the entrance of the flagellum the pigment of the organism was violently agitated, without, however, any disturbance of the outline of the organism. Soon all became quiet again and the period of quiescence lasted for about fifteen minutes, when a conical process began to appear at one margin of the organism, which, increasing in size, drew into itself most of the protoplasm, the pigment to a certain extent being gathered into the remainder. Finally most of the pigment was concentrated into a small round appendage, which remained attached to the end of what now had became an elongated fusiform body, which soon swam away with a gliding motion.

Have we not here, without much doubt, a sexual process in the organisms the result of which is the motile vermiculus? This is a process which we might have expected and which I am confident will be found to occur in the case of the human malarial parasites, although on account of the small number of organisms to be found in each slide in the latter case, the completion of the process will rarely be observed. It is entirely analogous to the sexual process as it occurs in the lower plants under unfavorable conditions. In Spirogyra and other related algæ, when their circumstances have become such that the ordinary reproduction by fission can no longer result in the successful prolongation of life, a more resistant body is formed by the conjugation of the adjacent cells of two filaments; but so long as conditions of growth are favorable we look in vain for this process. A similar conjugation is observed in many of the Ascomycete fungi and many other plants. In the animal kingdom we find it again and again, and I need only mention the pond Amœba as a familiar example.*

* Since the communication of this paper to the British Association for the Advancement of Science in Toronto on August 24, 1897, I have been much interested in the perusal of the article by Simond (*Annales de l'Institut Pasteur*, July 25, 1897, p. 545), in which he describes in certain coccidia a process analogous to that described here and suggests the probability of the occurrence of a sexual process in the malarial organism.

We can thus consider the two forms of adult organism found in the fresh blood as male and female, the granular female form containing more chromatophilic substance than the hyaline male form, which we may now say gives up its life in the production of four or more spermatozoa or flagella. Now, too, the mistake of thinking that the motile forms might arise from organisms in which the process of flagellation had proceeded less violently, is easily explained. Such organisms were simply the spherical granular forms surrounded by spermatozoa or flagella, which had arrived from the neighboring true flagellate forms and were undergoing the process of fertilization.

In view of all this it seems to me that we must look on the observations of Sakharoff with more confidence than has been accorded them by authors in general. Although I have stained with an excess of gentian violet the free flagella in the plasma and surrounding female spheres, I have not been able so far to confirm his statements by the application of staining reagents, but I feel assured that, were such nuclear staining successfully carried out, we should see in all essentials his clustered chromatic filament which, on breaking up into several filaments, forms, each with a certain amount of protoplasm, several flagella. His ideas as to the cause of the process are rather more hypothetical.

To return to the organism, immediately after fertilization, a process which when once recognized I have observed daily, it seems clear that the agitation of the pigment on the entrance of the flagellum is due merely to the mechanical motion of the wriggling flagellum, for it quiets down very soon. From this point on for ten to fifteen minutes it seems probable that, with suitable nuclear stains, one might demonstrate the coalescence of male and female nuclei, but I have not as yet done so. After a lapse of fifteen to twenty minutes the organism becomes motile, as above described, by the passage of the protoplasm into a conical process (Plate XII, Fig. 8), which gradually enlarges so as to contain the whole organism with the exception of a small spherical appendage into which most of the pigment is crowded (Plate XII, Fig. 9). The organism now appears quite fusiform, or at times egg-shaped, or even almost spherical, the anterior small end being free

from the pigment, which is distributed quite superficially over the posterior part. Toward the anterior part, but sometimes quite in the middle of the organism, there lies a fairly definite nucleus with well-marked nucleolus. This nucleus does not stain sharply, but is quite readily seen in fresh specimens, although no structure so definitely marked off by its different refractive index was observable in the adult male and female forms of the organism. The protoplasm retains the characteristic granular appearance seen in the female form.

Occasionally in beginning its movements away from the nucleus of the red corpuscle the organism leaves behind it part of the pigmented appendage—or it may possess two of these spherical appendages attached one behind the other, and in starting leave one of these behind to be broken down or engulfed by a leucocyte. The movement has nothing of the character of that of the flagellate forms, but is slow and even, the organism progressing invariably with the pointed end, free from pigment, forward. It can move in any direction readily, curving about obstacles and passing accurately between groups of corpuscles. Often it is seen to rotate continually on its long axis, at which times the superficial position of the pigment is readily seen. This rotation does not seem, however, to be in any way necessary to the forward motion. Again a third form of motion is observed in that a sort of peristaltic wavy constriction may repeatedly pass from anterior to posterior end of the organism, giving rise to very considerable distortions.

The forward progression of the organism occurs with very considerable force, as one may often be seen to thrust aside a group of red corpuscles. More often, however, they push directly through the obstacle, and it is interesting to observe that they can and do dash through the body of a leucocyte, tearing it apart, scattering its refractive granules into the plasma and often dividing its nucleus into two parts, thus leaving it to disintegrate. Passing on unharmed they attack masses of red corpuscles and leave those in their path as shadows of which soon only the nucleus is visible. This blanching of the corpuscles seems to become possible to them by the merest touch of their anterior pointed ends, which apparently puncture the capsule of the corpuscle and allow the hæmoglobin to escape into the plasma. Indeed,

in a thick slide of blood, a single organism may circle about and clear for itself a very considerable space in which there remain only shadows with the nuclei of corpuscles, and in some of the intense infections, which I have referred to, every slide of blood, after standing from thirty minutes to an hour, will show nothing but shadows, the organisms, very young and half-grown forms as well as segmenters, being often left free in the plasma. On one or two occasions these forms have been seen to put out pseudopods which attacked the corpuscles in a similar way.

In passing through a group of corpuscles it is frequently observed that the posterior end of the moving organism adheres to the corpuscles, and it may often be seen dragging three or four behind it. These soon become detached, however, and fall back, sometimes greatly distorted. In other cases the organism adhering to the corpuscle may draw out its capsule into a process, which gradually sinks back when the organism breaks away.

The ultimate fate and the true significance of these motile forms is difficult to determine, for in a slide, after being watched for more than one or two hours, the conditions become such that conclusions can scarcely be drawn as to the result under less artificial circumstances. Simple quieting down and final disintegration seem to be the end of many, but in some cases the organism was seen suddenly to stretch itself out by jerks into a greatly elongated form, sometimes dividing into segments connected by a fine thread. Generally in such cases the clear anterior end became separated from or connected only by a thread with the rest (Plate XII, Fig. 10), and often finally swam away by itself, leaving the rest to the leucocytes. This clear portion, however, was never seen to accomplish anything other than disintegration, although it was often watched with the idea that it might enter a corpuscle and set up a new cycle of existence.

It seems to me most reasonable to suppose that the motile form is the much sought resistant stage, or perhaps the first step in the production of such a stage. It seems probable that by the aid of such powerful means of locomotion and penetration it might make its way through a considerable obstacle of tissue to gain a new medium. This

of course might occur in several ways in the dead body subject to external violence. But in no case which I have examined post mortem did the organisms show any signs of life after the lapse of two to three hours, although the blood was still perfectly fluid in the vessels. In the living body, however, it seems that the intestine would form the most ready means of escape, and accordingly search was made in the fæces for such forms. In the birds at my disposal, however, in some cases the infection was so slight that results could hardly be expected, and in the cases where the infection was large, the presence of numerous echinostomes in the rectum rendered the mode of entry of the abundant blood corpuscles and organisms into the intestine doubtful. In dried smears from the contents of the small intestine, however, free organisms, free malarial pigment and one intra-corpuscular organism were found. More certain data might be expected from sections of the intestine with its contents, and examination of such sections showed immense numbers of organisms, mostly intra-corpuscular but sometimes extruded, in the capillaries of the stroma of the intestinal mucosa. In the mucous contents of the intestine were numerous *free* organisms, several of which exhibited the form described above as characteristic of the motile stage. The fact that the organisms were lying quite free in mucus, and that no corpuscles or nuclei of corpuscles were found, argues in favor of the idea that these organisms penetrated the mucosa after extrusion from the corpuscle. No organisms were found on the way through the epithelium. The inference that the organisms escape thus from the body seems plain, and further examinations of the intestinal contents obtained fresh immediately after death would be interesting.

In concluding this portion of the paper I may be allowed briefly to summarize what I have said.

Two types of organism occur so far as is known in the blood of birds. Minute morphological descriptions of these have been omitted in this paper. In the adult stage of one of these (Halteridium) there are certain differences between two forms which are thought to be sexual differences. Of these forms, one on extrusion from the corpuscle lies quiet and is soon approached and fertilized by a "flagellum" pro-

duced by the other form on extrusion from the corpuscle. The result of this fertilization is a motile stage which seems more resistant, and it is thought possible, in view of certain facts, that this form may make its way through the intestinal canal to the external world.

Reasoning by analogy it is thought very probable that the significance of the flagella in the human organism is similar to that given here for the organism of the birds.

THE PATHOLOGY OF HAEMATOZOAN INFECTIONS OF BIRDS.

The literature on the pathology of hæmatozoan infections is very meagre indeed. Danilewsky * speaks of the changes in the spleen and bone-marrow and describes macrophages with their varied contents. Kruse † made similar observations as to the spleen, bone-marrow, liver and lung, and Labbé ‡ also speaks in a general way of the gross changes in the organs. In the preceding paper on the subject, in this Journal (p. 103), prepared a year ago, I have given an account of the microscopic appearances of the various organs. I desire here to present a general summary of the more important pathological changes based upon the former observations and those made during the past summer.

Notwithstanding what has been said to the contrary, phagocytosis goes on very actively in the blood, as seen in a fresh slide. The leucocytes are seen to be contracted into small spheres on first examining the fresh drop of blood, but after about five minutes they begin to wander about and engulf whatever is foreign to the normal blood. These few minutes of inactivity are probably due to the shock of making the preparation. Both the eosinophiles and the rubinophiles (p. 113) show themselves to be very voracious. In the hardened tissues, however, these cells, which are readily recognized by their oxyphilic granulations, are very seldom seen to contain any pigment. On the other hand the cells most actively phagocytic there are principally the endothelial and connective-tissue cells. The deposition of pigment,

* *Ann. de l'Inst. Pasteur*, iv (1890), Parasitologie comparée, 1889.

† Virchow's *Archiv*, cxx and cxxi.

‡ *Arch. d. zool. expér.*, 1894.

together with the remains of infected corpuscles, organisms, etc., forms the main pathological change in the organs, and in order to make this change plain I shall begin by describing an organ in which it is most simple.

In the *kidney*, as in all the other organs, the capillaries and blood-vessels generally show a great number of infected corpuscles and some organisms extruded from the corpuscle. The epithelium of the tubules is in general fairly well preserved, although there are numerous instances of a hyaline degeneration of the cells. The glomeruli show nothing except the organisms within their capillaries. It is in the connective-tissue skeleton of the kidney with its contained capillaries that we see the main results of the infection. Many of the endothelial cells of the capillaries are much swollen and contain masses of pigment, yellow as well as malarial. Besides these there are cells belonging to the connective-tissue framework, which have become storehouses of pigment—large cells with large round or oval vesicular nucleus, which have become so loaded with pigment, etc., as to be very conspicuous in the section. No epithelial cells have been seen invaded.

In the *intestine* the connective-tissue cells of the stroma are phagocytic and sometimes contain considerable masses of pigment. Similar cells occur also in the muscular wall imbedded among the muscle fibres, the pigmentation being deepest in the subperitoneal portion. The endothelial cells of the vessels in this tissue are also pigmented. The epithelial cells of the mucosa never show foreign contents.

In the *bone-marrow* we have, besides fat cells and blood-vessels, cells large and small, which are generally spherical, with round nucleus and containing basophilic granulations, and other rounded cells and nuclei of various sizes with homogeneous protoplasm, all supported by a loose skeleton of connective tissue, made up of irregular, sometimes branching cells with pale ovoid nucleus. Of all these varieties of cells only the last are seen to be pigmented. Other large round cells do occasionally occur in the bone-marrow, however, which correspond closely to the red-corpuscle-carrying cells of the human spleen; these often contain several red corpuscles, some of which are infected.

In the *lung*, *brain*, and *pancreas*, although the capillaries contain many organisms, hardly any pigment deposit can be seen.

The thyroid and *adrenal*, however, show a very considerable pigmentation exactly corresponding in nature with that described for the kidney, the pigment being deposited in the endothelial and connective-tissue cells of the interglandular tissue, the secreting cells escaping. The adrenal, however, differs in that red-corpuscle-carrying cells occur in its small vessels together with large free pigmented cells with hyaline protoplasm and vesicular nucleus. These resemble precisely the macrophages in the spleen, to be described presently. In the tissue adjoining the thyroid gland there are large spaces lined by epithelioid cells, many of which contain pigment masses, or even several infected corpuscles. Lying free in these spaces are many large red-corpuscle-carrying cells together with numerous red corpuscles, leucocytes and cells desquamated from the walls, surrounded by a fine network apparently of coagulated fibrin. The nature of these spaces I do not know more exactly.

In the *pectoral muscle* in one case there was a greyish opaque spot which on section showed a mass of necrotic material lying in the muscle and surrounded by richly cellular tissue, which was quite deeply pigmented. The neighboring muscle fibres were much fragmented, with a proliferation of nuclei reminding one of that seen in the muscle in cases of trichiniasis. The tissue about the necrotic material had some of the characters of granulation tissue and was quite vascular, the elongated cells with large vesicular nucleus being the pigmented ones. Scattered about in this tissue were numerous giant cells with necrotic centre and many nuclei, such as are seen in miliary tubercles. Some of these were very large and without definite boundaries. The large mass of necrotic material was closely bordered by groups of clustered nuclei, suggesting that it had arisen from the coalescence of many giant cells. Through it there was scattered a good deal of malarial pigment. Whether all this diseased tissue in the muscle was due to the action of the hæmatozoon is doubtful; it seems to me much more probable that the deposit of malarial pigment throughout it was entirely secondary to its formation by some other process.

In the *liver* we have often a very extensive deposit of pigment. The liver cells themselves are never phagocytic, but the endothelial cells of the capillaries are often greatly swollen by their load of pigment and debris. In the blood-vessels, especially the branches of the portal vein, we find lying free the large macrophages, such as were seen to occur in the adrenal. These sometimes are so large as to contain masses of pigment equalling in size three or four liver cells. There are other phagocytic cells with large irregular nucleus which seem to lie between the liver cells and the endothelium and, although at first sight they have somewhat the appearance of liver cells, I am inclined to think them analogous to Kupffer's cells.

It is in the *spleen* that these processes reach their acme. This organ is almost always greatly swollen and blackened, the deposit of pigment being so disposed as to show a black network on section. The vascular pulp of the spleen is arranged in bands forming a network in whose meshes lie the lymphoid elements. The pigment deposit is confined to these vascular bands beginning at the margins, where they are sharply marked off from the lymphoid tissue, and gradually involving most of their width. The bands appear to be made up of a spongy reticulum of endothelial cells, between which the blood flows. These cells are more closely arranged at the boundaries of the lymphoid tissue, where they become conspicuous early in the infection by their pigmentary contents. In more intense infections a great majority of the fixed cells making up the pulp become loaded with pigment, and thus give rise to the black network mentioned above. It seems probable that such loaded cells may be washed into the blood stream and constitute the so-called macrophages. These have been mentioned as occurring in the blood of the liver and adrenal, and they are also seen in numbers in the veins of the spleen. They are cells varying greatly in size but generally of a somewhat rounded outline and with one or sometimes two vesicular nuclei; their protoplasm is generally very clear and is seen to contain a great deal of foreign material—yellow masses of pigment as well as the balls, irregular masses, and granules of malarial pigment, broken-down blood corpuscles and disintegrated nuclei, together with shrunken parasites. The last sometimes occur

in great numbers and form the only contents of the cell. In the spleen as elsewhere the endothelial cells of the vessel walls are often seen to be loaded with pigment. Red-corpuscle-carrying cells are not frequently seen.

The pigment found in all these cells is not exclusively that formed by the parasitic organisms, as has been mentioned. A large part of it is the iron-holding compound, or mixture of compounds, grouped under the name hæmosiderin, which are formed from the hæmoglobin set free on the rupture of the infected corpuscles.

We have, then, as the result of the hæmatozoan infections the production of one form of pigment by the parasites from the hæmoglobin, and of another form by the tissues of the host from the hæmoglobin set free into the blood. These solid pigments are stored up in the various organs, but more particularly in the spleen and liver, in cells belonging exclusively to the connective-tissue group which also engulf the infected corpuscles, broken remains of corpuscles and shrunken dead parasites. The anatomical changes, therefore, differ very slightly from those seen in the human being in malarial infections, so well described by Bignami * and by Barker,† the greatest difference perhaps lying in the less important part played by leucocytes in the case of the bird.

Finally, I wish to express my indebtedness to Dr. Thayer, of the Johns Hopkins Hospital, for his many kindnesses to me while engaged in this work, which was undertaken at his suggestion.

ADDENDUM.

Since the preparation of this article for the press I have examined the blood of a woman suffering from an infection with the æstivo-autumnal type of organism, in which a great number of crescents were to be seen. These in a freshly made slide of blood, with very few exceptions, retained their crescentic shape for only a few minutes. They soon drew themselves up, thus straightening out the curve of the crescent while shortening themselves into the well-known ovoid form.

* Bignami, *Bull. d. r. Accad. med. di Roma*, xix (1892-3).
† Barker, *Johns Hopkins Hospital Reports*, v (1895).

After the lapse of 10 to 12 minutes most of them were quite round and extra-corpuscular, the "bib" lying beside them as a delicate circle or "shadow" of the red corpuscle.

After 20 to 25 minutes certain ones of these spherical forms became flagellated; others, and especially those in which the pigment formed a definite ring and was not diffused throughout the organism, remained quiet and did not become flagellated. In a field where an example of each form could be watched, the flagella broke from the flagellated form and struggled about among the corpuscles, finally approaching the quiet spherical form; one of them entered, agitating the pigment greatly, sometimes spinning the ring about. The rest were refused admission, but swarmed about, beating their heads against the wall of the organism. This occurred after 35 to 45 minutes.

After the entrance of the flagellum the organism again became quiet and rather swollen, but although in the two instances in which this process was traced the fertilized form was watched for a long time, no form analogous to the "vermiculus" was seen.

This process has also been observed once by Drs. Warfield and Pancoast of the Johns Hopkins Hospital in another case of infection with the æstivo-autumnal parasite. It is evidently for the human being what was foreshadowed by the organisms of the bird.

DESCRIPTION OF PLATE XII.

All of the figures are of the Halteridium form of the parasite.

Fig. 1.—Adult granular, or female form.

Fig. 2.—Adult hyaline, or male form.

Fig. 3.—Adult female preparing for extrusion from the corpuscle.

Fig. 4.—Adult male preparing for extrusion from the corpuscle.

Fig. 5.—Adult female extruded and lying beside the nucleus of the corpuscle.

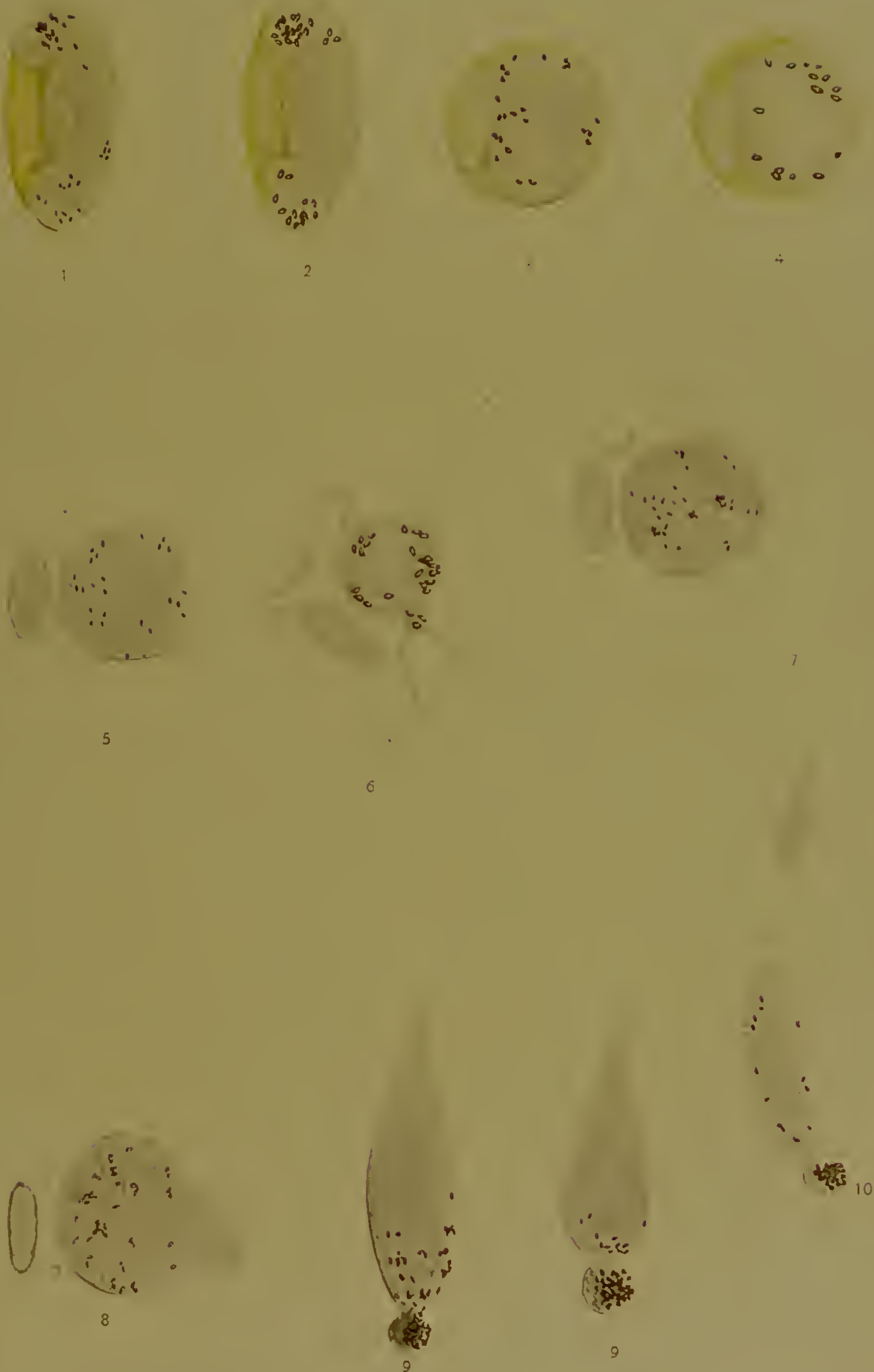
Fig. 6.—Flagellate form.

Fig. 7.—Process of fertilization.

Fig. 8.—First stage in formation of motile form.

Fig. 9.—Fully developed motile form or vermiculus.

Fig. 10.—Fully developed motile form in process of disintegration.


1
2
4
5
6
7
8
9
9
10